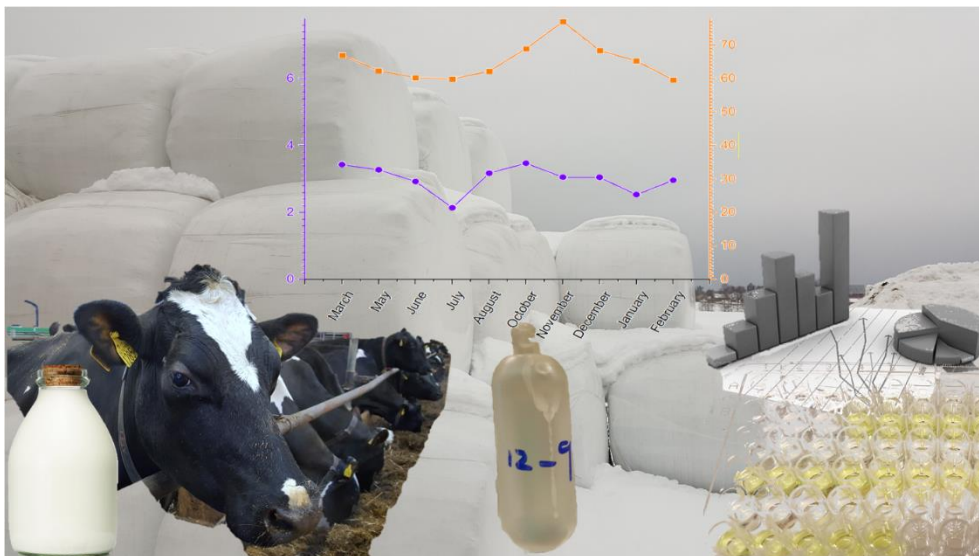


Plasmin, plasminogen, protein and somatic cells variation of bulk milk

Impact of breed, milking system and production months

Shishanthi Jayarathna



Department of Molecular Sciences

Master's thesis • 30 hec • Second cycle, A2E

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Abstract

During a period of one year, milk samples were collected from 45 different farms in Northern Sweden. Plasmin (PL), plasminogen (PG), somatic cell count (SCC) and total protein (TP) content were investigated in bulk milk received at the dairy. The composition of bulk milk samples was related to major farm and production variables (breed, milking system and production months). SCC and TP were measured in whole milk while, PL and PG analyses were performed on serum fraction in duplicates obtained by ultracentrifugation of defatted milk. PL and PG derived activities were analyzed by spectrophotometric method using multi-mode microplate reader at 37°C. Urokinase (49.5 plough units) was used as PG activator to measure the total proteolytic activity of PL and PG. The PG derived activity was not influenced by the breed class. The farms having mixtures of breeds had higher ($P < 0.05$) PL activity as compared to farms having Holstein, Swedish Red and both Holstein, Swedish Red and their crosses. PL and PG derived activities had no influence ($P > 0.05$) from the milking system and production month for the considered farms. SCC varied with breed, lowest SCC ($P < 0.05$) was reported for Swedish Red breed and highest ($P < 0.05$) was reported for mountain breeds. Higher ($P < 0.05$) SCC counts were observed in AMS farms compared to CMS farms. SCC varied slightly according to the production month. Lower ($P < 0.05$) TP content was recorded for mountain breeds than for Holstein, Swedish red and farms having mixture of breeds. Jersey breed showed higher ($P < 0.05$) TP content than other breeds. Higher ($P < 0.05$) bulk milk TP content was observed for CMS than AMS farms. The mean TP content varied ($P < 0.05$) with the production month, with higher amount in October, November and December than in June, July and August. There was no clear trend or correlation of PL activity and PG derived activity with bulk milk TP or SCC.

Keywords: plasmin, plasminogen, production month, somatic cell count, total protein, cow breed, milking system

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Abbreviations

PL	Plasmin
PG	Plasminogen
PA	Plasminogen activator
PAI	Plasminogen activator inhibitor
PI	Plasmin inhibitor
SCC	Somatic cell count
TP	Total protein
CMS	Conventional milking system
AMS	Automatic milking system
pNA	p-nitroanilide

1 Introduction

Dairy proteins are crucial for processing and determining the final product characteristics. The majority of the protein fraction is consisting of casein (80%), which is economically important for dairy processors. Proteolytic enzymes are mainly in favour of casein protein groups. Proteolytic activity is mediated through proteases and these proteases can be indigenous or exogenous. Indigenous serum protease, plasmin (PL) is the principal proteolytic enzyme in bacteriologically high quality milk. PL hydrolyses the β -casein in to γ_1 , γ_2 , γ_3 casein and proteose-peptones (Ardo, 2001). Regulation of PL activity and identifying the variation of PL level is crucial to control the quality of processed dairy products. PL activity may be beneficial or detrimental, depending on the type of dairy product and the extent of hydrolysis by PL (Ismail and Nielsen, 2010). In case of cheese, desirable flavour and texture development occurred as a result of breakdown of protein by PL (Fox, 1989). According to Bastian et al. (1997), PL is mainly responsible for ripening of specific cheese varieties.

However, as reviewed by Chavan et al. (2011), for dairy products such as UHT milk, concerns on the PL activity of raw milk is important since it can cause product defects such as development of bitter flavour and gel formation upon storage. Such defects reduce the shelf life and marketability of the products. PL might be involved in age related gelation of UHT milk by hydrolysing casein-casein and casein-calcium phosphate interaction sites of the casein micelles (Rauh et al., 2014). Therefore, understanding the proteolytic activity in relation to PL in milk is a greater interest for dairy producers and researchers.

It is known that plasminogen (PG) is the zymogen form of PL which is activated by plasminogen activators (PA). PL and PG are associated with casein micelles and therefore, close proximity of proteolytic enzyme and substrate ensure the efficient hydrolysis. PL hydrolyse β -casein and cause degradation of the casein micelle. Thus, understanding the influences of PL on the different group of casein proteins are of interest for researchers. Recently, many studies have been undertaken to elucidate the importance of PL in milk. However, published

materials concerning proteolytic activity in milk as affected with on-farm factors are limited. Thus, the present study attempts to fulfil the scientific knowledge gap within this arena: the effect of on farm factors and production month on PL and PG derived activities of raw milk.

Bulk milk samples intended for production of long ripened cheese were collected from northern Sweden during a period of one year from 45 different farms. The farms were different from each other in many aspects, such as the breeds, milking systems, and feeding. The current study is a part of a larger project which aims to investigate and link the effect of on-farm factors (farm variabilities) on raw milk quality and sensory attributes of long ripened cheese. Therefore, as long term perspectives are concerned, it is anticipated to establish future links between PL in raw milk and final quality of the cheese. Therefore, it was hypothesized that on farm level production factors (i.e., breed and milking system) and production month are connected to raw milk quality, significantly affecting the PL, PG, TP and SCC of bulk milk.

1.1 Objective and hypothesis

The aim of this study was to investigate if on farm factors (i.e., breed, milking system and production month) significantly affect the plasmin, plasminogen, total protein content and somatic cell count of the bulk milk from 45 different farms in northern Sweden collected during one year. It was hypothesised that

- The bulk milk plasmin activity and plasminogen derived activities are affected by the breed, milking system and the production month.
- The bulk milk total protein content is affected by the breed, milking system and the production month.
- The bulk milk somatic cell count is affected by the breed, milking system and the production month.
- The bulk milk plasmin and plasminogen activity is correlated to the bulk milk total protein content.
- The bulk milk plasmin and plasminogen activity is correlated to the bulk milk somatic cell count.

2 Literature Review

2.1 Milk composition

Milk is synthesized in the mammary gland of mammals. It is nutritious and comprises of different components, which are important for the growth of new born off-spring. Cow's milk composition is affected by breed, lactation, feeds, seasons and management practices (Auldist et al., 1998, Lucey, 1996 and O'Callaghan et al., 2016). In general, cow's milk contains approximately 87% of water, 3.3% of protein and other solids (Walstra et al., 1999).

Milk proteins and peptides are responsible for the most physiological functions of milk. Such proteins and peptides include enzymes, enzyme inhibitors and activators, immunoglobulins, growth factors, hormones and anti-bacterial agents (Fox and McSweeney, 2003). The essential and most important dairy proteins with regard to dairy food processing are caseins (80%) and whey proteins (20%). Caseins exist in milk serum as hydrated colloidal aggregates with calcium phosphate nanoclusters, which is referred to as the casein micelle (Dalglish, 2011). As reviewed by Fox and McSweeney (2003), 80% of the milk proteins are caseins. The casein precipitates from milk near pH 4.6 and it becomes slowly insoluble when milk is heated above 120 °C (Walstra et al., 2005). In the casein micelle structure, α_s and β caseins are arranged internally and κ -casein resides on the outer periphery. The κ -casein stabilizes the casein micelle in milk serum by avoiding coagulation through electrostatic and steric stability (Horne, 2014). α_{s-1} casein is the major casein in bovine milk and it is responsible for 38% of the total casein followed by 34% of β -casein. κ -casein and α_{s-2} casein make up 15% and 10% of total casein, respectively (Fox et al., 2016).

The whey protein fraction comprises approximately 20% of the total protein in bovine milk. Whey proteins are dissolved in serum, therefore called serum proteins (Fox and McSweeney, 2003). β -lactoglobulin is the major serum protein, while α -

lactalbumin is a coenzyme that contributes in the synthesis of lactose. Whey proteins are globular proteins and have higher hydrophobicity.

2.2 Milk as a raw material for dairy industry

Milk of all species is considered as a highly nutritious and well-balanced food. Hence, humans have consumed milk from other species for more than 8000 years (O'Mahony and Fox, 2014). Eventhough sheep and goats were the earliest animals to be domesticated (Fox et al., 2016), today cattle is the major animal species that is utilized for milk production and it accounts for 84% of the total milk production (O'Mahony and Fox, 2014). Apart from the rich nutritional profile, milk is interesting in terms of containing several bioactive compounds that are important for human health (Park, 2009). However, milk is a highly susceptible food for microbial spoilage. To withstand this problem humans have developed many milk products that are more stable than raw milk. The principal categories of such products include beverage milk, cheese, milk powders, concentrated milks, fermented milk products, butter, ice cream, infant formula, creams, protein rich products and lactose (O'Mahony and Fox, 2014). Some of the above categories are very diverse. For example, today there are around 1400 of cheese varieties (O'Mahony and Fox, 2014). The raw milk may be utilized for specific products based on the quality attributes of raw milk that may influence on the final product quality. For example, factors such as breed of the cow, seasonal and lactational variations, nutritional factors, milk production methods including hygiene, storage and collection methods will all influence on the final product quality of cheese (Fox et al., 2016).

2.3 Proteolytic activity of milk

Proteolytic activity of milk is a result of external and internal factors. Proteases produced by bacteria are an external factor contributing to milk protein degradation (Mitchell and Ewings, 1985). Indigenous enzymes such as PL, PG and lysosomal proteinases of somatic cells are considered as internal factors that contribute to proteolysis of milk proteins. Somatic cells contain proteolytic enzymes such as elastase, collagenase and cathepsin B, D, G, H and L (Kelly and McSweeney, 2003). Furthermore, somatic cells contribute to milk protein degradation by containing a plasminogen activator (PA) that activates PG into PL (White et al., 1995). As reviewed by Kelly et al. (2006), both PL and lysosomal proteinases of somatic cells are involved in hydrolysing caseins in milk. Cathepsin D is lysosomal proteinase with low optimal pH and stemming from its inactive

zymogen, procathepsin D. Cathepsin D has specificity similar to that of chymosin for hydrolyzing α_{s-1} -casein and β -casein as reviewed by Hurley et al. (2000). Cathepsin B has broader casein specificity, however, will cleave the Phe23–Phe24 bond of α_{s-1} -casein in a similar way as PL or chymosin (Considine et al., 2004). Elastase will cleave the β -casein with broader specificity and is probably present in the milk from mastitis cows (Considine et al., 1999).

2.4 Proteolytic enzymes

2.4.1 Plasmin (PL)

PL is a blood enzyme which has been studied extensively as an endogenous protease in bovine milk (Ismail and Nielsen, 2010). PL, (EC 3.4.21.7) is a serine proteinase and also referred as milk alkaline proteinase from bovine plasma (Chen et al., 2003). Kelly et al. (2006) reviewed PL as one of the major proteolytic enzymes found in milk, with greater interest in processing and storing of raw milk. Bastian and Brown, (1996) reviewed that PL mainly acts on the milk caseins and degrade β , α_{s-1} and α_{s-2} caseins to γ -casein, proteose peptones and λ -casein, respectively. PL is a heat stable enzyme (Sharma et al., 2014) and pH dependable (Dulley, 1972). The association of PL to casein micelles is mediated by the pH. The optimal pH range for activity of PL is pH 7.5-8.0 and optimum temperature for PL activity is 37 °C (Fox, 1981). Also, as reviewed by Ismail and Nielsen, (2010), apart from pH, PL activity may alter with several other factors such as mineral content, whey proteins, thermal processing and storage conditions. The whey fraction contains plasmin inhibitors (PIs) and plasminogen activator inhibitors (PAIs). According to Richardson (1983b) PIs and PAIs are heat liable and thermal processing will therefore influence the PL system by influencing the PIs and PAIs. Further, the heat inactivation efficiency of PL is directly linked with the presence of β -lactoglobulin since it accelerates the thermal inactivation of PL (Denis et al., 2001). Storage temperature can affect the PL activity by several ways. For example, cold storage causes β -casein to become more soluble and it facilitates easy access for PL. Prolonged storage at room temperature causes autolysis of PL in UHT milk and non-fat dry milk (Ismail and Nielsen, 2010).

2.4.2 Plasminogen (PG)

PG is a blood proenzyme and it associates with casein in milk. It is a glycoprotein that is activated to PL in the presence of PG activators (PA) by cleaving a peptide

bond (Fox and McSweeney, 2003). PG is a very heat resistant proenzyme (Walstra et al., 2005). According to Chavan et al. (2011), considerable proportion of PL activity of milk is emerged by PG. Neither PL nor PG in milk stem from the milk and most of the PG and some of the PL are transferred to the milk via paracellular routes (Stelwagen et al., 1994).

2.5 Plasmin system

PL is the major component in a complex that consist of its inactive form PG, PA, and inhibitors (Ismail and Nielsen, 2010). This complex is known as the plasmin system (PL-system) and is illustrated in Figure 1. The zymogen form (PG) of PL is the predominant component in the plasmin system. This can be converted to PL by PAs (Grufferty and Fox, 1988). At least four types of PAs are found in cows' milk (Deharveng and Nielsen, 1991). There are two categories of PAs: urokinase-type and tissue-type and they were cloned by Ravn et al. (1995).

Plasminogen activator inhibitor (PAI) and plasmin inhibitor (PI) are also important components in PL-system that act antagonistically on the PAs and PL, respectively. As reported by Politis (1996) α 2-antiplasmin and α 2-macroglobulin are the important PIs. Further, PAI 1 and PAI 2 are known as important PAI s.

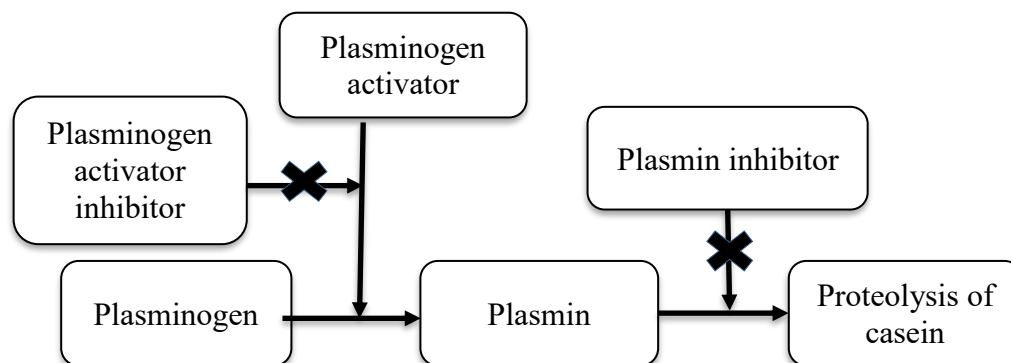


Figure 1. The PL System in bovine milk (Reproduced from Ismail and Nielsen, 2010)

2.6 Measurement of PL activity in milk

Multiple assay methods are commonly employed to study and quantify the indigenous milk enzymes, and fluorimetric and spectrophotometric assay procedures have been extensively used to study the PL activity (Kelly et al., 2006). This includes measuring the amount and rate of formation of the enzymatic

products. Igarashi (1989), described a method to study the PL system by determining the γ -casein which is an end product of PL hydrolysis of casein.

Wang et al. (2006) reported the suitability of using fluorescent label techniques for characterization of bovine milk proteinases. These authors used Alexa fluor 594 labeled PG to study the concentration and location of PG in stimulated bovine milk and concluded that the labelled PG was comparable to the native PG in milk.

Use of a reporter enzyme to measure the proteinases was described by Andrews (1982). The casein was immobilized on an insoluble particle (Sephacrose) which was then linked to a reporter enzyme. Due to the proteolytic activity of PL, the casein is hydrolysed releasing the reporter enzyme from insoluble particles. Incubation thereafter allowed the reporter enzyme to act on its own substrate and release the end products in a similar rate as the PL acts on casein.

Collin et al. (1988) developed an ELISA technique to determine the PL and PG concentration in milk products. The values were compared with the values obtained by fluorometric assay. However, the authors reported an overestimation of the values by 2.5 fold adopting the ELISA technique.

Spectrophotometric assay of PL and PG using chromogenic substrate was developed by Rollema et al. (1983). The chromogenic substrate, H-D-valyl-L-leucyl-L-lysyl-4-nitroanilide (S2251) was used, with 4-nitroalanine, absorbing light at 450 nm, being released from the substrate due to PL action. Similarly, fluorogenic substrates are also in use to determine the PL and PG derived activities of milk (Saint-Denis et al., 2001). However, these chromogenic and fluorogenic measurements have some drawbacks. For example, even though the methods include little sample preparation, influence of other proteinases such as bacterial proteinases cannot be avoided in measurements (Bastian and Brown, 1996).

2.7 Factors affecting PL and PG derived activities

2.7.1 On farm factors

Bastian et al. (1991a) concluded that the PL activity is greatly influenced by the lactation number compared to the other investigated factors such as breed, stage of lactation and season. In agreement, Bastian and Brown (1996) reviewed that PL activity increases with lactation number. Moreover, the authors argued that PG derived activity is affected only by stage of lactation and season. Politis et al. (1989) and Fantuz et al. (2001) concluded that the stage of lactation also increase the PL activity. In agreement, as reviewed by Bastian and Brown (1996), PL activity is affected by stage of lactation and it increases at the end of the lactation.

The authors further argued that it may be due to increased entrance of PL from mammary epithelium as the cow advanced with the lactation. The increased concentration of PL and PG with the progression of lactation was due to increased leakage of these enzymes from serum to milk (Stelwagen et al., 1997). Hence the same authors argued that this increment of proteolytic enzymes is due to losing the integrity of tight junctions between mammary epithelial cells.

As reported by Richardson (1983), PL and PG derived activities of milk vary according to the breed of the cow. The different casein contents of milk may cause milk from different breeds to exhibit varying PL activity (Bastian et al., 1991a). Richardson (1983) reported that the PL activity of milk from Holstein cows was higher than milk from Jersey cows. In contrast, Fantuz et al. (2001) concluded that the PL and PG derived activities and PA activities do not significantly vary between Jersey and Holstein cows. The authors further reported that the ratio of PG/PL is affected by the breed where a lower ratio was reported with regard to the Holstein breed. The ratio of PG/PL is inversely related to the proteolytic activity of bovine milk as reviewed by Sorensen et al. (2001) and the ratio of PG/PL is independent of milk volume (Stelwagen et al., 1994).

Kelly et al. (1998) reported that milking frequency has an effect on the PL and PG derived activities in milk from Holstein Frisian cows. Further, the PL and PG derived activities were significantly higher in cows having reduced milking frequency. Sorensen et al. (2001) reported that decreased storage time of milk in thrice daily milking leads to reduction in the PL activity and stated that this could be due to reducing the time available for converting the PG to PL. According to the same authors, PL and PG derived activities were decreased in thrice-daily milking and increased in twice-daily milking. These findings are in agreement with Stelwagen et al. (1994) who reported that less frequent milking lowered the PG/PL ratio.

The PL and PG derived activities of milk vary due to the milking system. As a consequence of structural changes of dairy farming and labour shortages, automated milking systems (AMS) are emerging compared to the conventional milking systems (CMS). AMS are shown to produce milk with good quality and safety and therefore, have gained popularity in dairy farming (De Koning, 2010). Milking frequencies and interval of two successive milking differ between AMS and CMS. As reviewed by Hovinen and Pyörälä (2011), cows in AMS milk more frequently than cows in CMS. Further, they highlighted that in CMS, clinical mastitis conditions can be easily detected as compared to AMS. The study conducted by Abeni et al. (2008), to evaluate the effect of CMS and AMS on PL and PG derived activities, reported that the PG and total (PL+PG) activities were lower in AMS as compared to CMS.

The stocking density of cows also has an effect on the milk PL activity. Increasing stocking density causes increment of the PL of milk (O'Brien et al., 1999).

The diet of the cows also affects the PL and PG activities of milk. According to the study conducted by O'Brien et al. (1999), restricted grass supply and concentrate supplementation leads to elevated levels of PL activity of milk.

2.7.2 Environmental factors

Both the PL and PG have seasonal fluctuations (Bastian et al. 1991a; Politis et al. 1989). Bastian et al. (1991a) reported that the PG derived PL was highest during Autumn and Winter. Politis et al. (1989) also reported the same with regard to the PL activity.

The proteolytic activity of PL and PG varies in products, such as UHT milk, that was produced from milk from different seasons. A study done with UHT milk reported that the total proteolytic activity resulted from the contribution of PL and PG was higher in UHT milk manufactured using winter milk than that of summer milk (Garbowska et al., 2010).

2.7.3 Processing

PL activity of milk is highly affected by the processing conditions. As reviewed by Ismail and Nielsen (2010), PL activity is mainly influenced by storage conditions of milk, processing conditions and activity of bacterial proteases.

Thermal processing influences the PL and PG derived activities. The effective inhibition of PL related proteolysis can be achieved by preheating the milk to 90 °C for 30-90 s (Newstead et al., 2006). A study concluded that the pasteurization of bovine milk at 72°C for 15 s caused to decrease both PL and PG derived activities by 10% while commercial ultrahigh temperature sterilization completely ceased the PL activity and reduced PG derived activity by 90% (Korycha-Dahl et al., 1983). However, in contrast, Richardson (1983b) reported that the pasteurization of milk (72°C for 15 s) causes the PL activity to elevate due to inactivation of PAIs. In agreement, Walstra et al. (2005) reviewed that this is due to the inactivation of the urokinase inhibitor due to low pasteurization conditions. Ismail and Nielsen (2010) also reported that although the further heat treatments will reduce the PL activity, the pasteurization conditions cause to increase the PL levels and PG activation due to inactivation of PI and PAIs. The authors suggested that the interaction of PL, PG and PA with denatured whey protein may be the reason to lower the PL levels upon further heat treatment. Further, Bastian and

Brown (1996), reviewed that PL does not inactivate at many UHT treatments even though it cause gelation of the UHT treated milk.

Salting has an effect on PL activity. PL hydrolysis of β -casein (Bastian et al., 1991b), and activity of PL may vary significantly depending on salting procedures during cheese making (Fox, 1989). Grappin et al. (1993) cited that the highest PL activity on β -casein was observed at a concentration of 2.3% of NaCl.

However, defatting of milk and removal of the fat fraction do not affect the PL or PG activity since the fat portion contains very low amount of PL and PG compared to the PL and PG associated with the casein (Hofmann et al., 1979). Rauh et al. (2014) also reported that the PL activity of the milk is not influenced by skimming.

2.7.4 Mastitis

Mastitis is known as an inflammation of the mammary gland caused by an infection and therefore, it has adverse impacts on milk quality. Mastitis causes an increase in the number of somatic cells in the milk. The PL activity of milk increases with increasing somatic cell count (SCC) (Walstra et al., 2005) and therefore, mastitis milk have higher PL activity than normal milk (Grieve and Kitchen, 1985). Mastitis also causes an increase in PL by increasing the influx of PL from blood and increment of PG activation with the advancement of lactation (Politis et al., 1989a). Further, Bastian and Brown (1996) reviewed that higher PL activity in mastitic milk may be due to both PA and proteolytic enzymes from somatic cells. Schaar and Funke, (1986) reported that the higher release of PL and PG from casein micelle is associated with mastitis milk. According to Politis et al. (1989b), the higher permeability of the active enzyme through mammary gland epithelium is the major factor that contributes for the higher PL activity in mastitis milk.

2.7.5 Somatic cell count (SCC)

SCC of milk, which is measured as the number of total cells per mL is an indicator of both udder health and quality of milk. As reviewed by Boutinaud and Jammes (2002), the predominant somatic cell type in most of the species is leukocytes such as lymphocytes, polymorphonuclear neutrophils and macrophages. However, the proportion of cell types differs according to the species. According to the authors, the normal SCC level of bovine milk is 7.5×10^4 cells/mL with macrophages contributing mostly to the SCC (by 61%). Through a study performed with goat milk, Dulin et al. (1983) concluded that intramammary infection, stage of lactation and number of lactation can influence the level of SCC in milk. As reviewed by

Boutinaud and Jammes (2002), the level of SCC rises as the stage of lactation progress. Further, SCC is affected by factors such as animal species, milk production level, the individual and environmental factors as well as management practices (Rupp et al., 2000).

The SCC and proteolytic activity of milk are directly related. A study conducted by Le Roux et al. (1995), concluded that proteolysis takes place in milk with SCC as low as 250,000 cells/mL.

As reviewed by Li et al. (2014), somatic cells have a number of endogenous enzymes which are released in to the milk and among them the proteinases (i.e., cathepsins, elastase, and collagenase) are widely studied. Other than proteases, somatic cells contain enzymes such as lipases (i.e., lipoprotein lipase), oxidases (i.e., catalase and lactoperoxidase) and glycosidases (i.e., lysozyme).

Li et al. (2014) reported that the endogenous enzymes of somatic cells have an influence on the other milk indigenous enzymes. For example, the PL activity of milk is influenced by the PAs from somatic cells. Moreover, studies done with milk other than cows' milk revealed that the PL activity is highly influenced by SCC (Albenzio et al., 2004). For example, a study done with ewes' milk reported a higher PL activity in milk having higher SCC ($>1 \times 10^6$ /mL) than in normal milk (SCC $<5 \times 10^5$ /mL) (Albenzio et al., 2005).

3 Materials and Methods

3.1 Collection of samples and labelling

Bulk milk samples were collected from 45 different dairy farms in northern Sweden during one year. Milk was sampled on a monthly basis by the tanker driver in connection with the regular collection of the raw milk at each farm. Bulk milk was stirred for two minutes before the samples were obtained. Milk samples were transported to the dairy in sterile plastic flasks. The milk was stored at 4°C at the dairy overnight and sent to the Swedish University of Agricultural Sciences (SLU). The samples were sent in plastic insulated containers with cooling blocks. The analysis of PL and PG derived activities of the milk samples was performed at the Åse Lundh Laboratory at the Department of Molecular Sciences, Uppsala Bio Center at SLU, and data referring to the analysis of SCC and total protein in milk samples were performed at Eurofins Steins Laboratorium AB (Jönköping, Sweden).

3.2 Sample preparation

The collected milk samples were defatted by centrifugation (Himac CT15RE, Hitachi Koki Co., Ltd.) at 10 000 g at 4°C for 10 minutes. The defatted milk samples were transferred to the Eppendorf safe lock tubes (Eppendorf, Germany) and stored at -80 °C for further analyses.

3.3 Preparation of the plasmin buffer

Plasmin buffer was prepared as described by Saint-Denis et al. (2001), with 20 mM ϵ -aminocaproic acid (EACA) (Sigma-Aldrich, China), 53 mM Trizma buffer

(Sigma-Aldrich, USA) and 117 mM NaCl (Merck KGaA, Darmstadt, Germany) prepared in 2 liters of distilled water. pH was adjusted to 7.4 using 5 M NaOH (Sigma-Aldrich, USA) and a pH meter (PHM210, Standard pH meter, MeterLab®, Germany). The buffer was stored at room temperature (20 °C).

3.4 Isolation of PL and PG

Isolation of PL and PG was performed according to the method described by Korycha-Dahl et al. (1983) with minor modifications. The milk samples were thawed at room temperature and vortexed (Vortex Genie2, Bergman Labora AB, Sweden) for 30 seconds. Milk (320 µL) was mixed with 4680 µL of plasmin buffer in 15 mL Falcon tubes (Sartedt, Germany). Falcon tubes were vortexed and incubated at room temperature for 2 hours to extract the PL and PG by dissociating from casein micelle. Korycha-Dahl et al. (1983) described that the EACA, which is a lysine derivative, will dissociate PL and PG from casein by binding to the lysine binding sites of PL and PG. The NaCl of the plasmin buffer will also contribute to the dissociation of PL and PG from casein (Saint-Denis et al., 2001). The milk serum fractions containing PL and PG were then separated by ultra-centrifuging the samples (Op-tima MAX-XP, Beckman Coulter, Inc., Bromma, Sweden) using RP55T angle rotor, 12 mL×12 at 4 °C For 1 h at 100 000×g. The milk serum fractions were then transferred to 2 mL Eppendorf safe lock tubes (Eppendorf, Germany) and stored at -20 °C.

3.5 Measuring of PL and PG derived activities

PL and PG derived activities of milk were measured according to the method described by Korycha-Dahl et al. (1983) and modified by de Vries et al. (2016). A spectrophotometric method using a multi-mode microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany) was used to determine the end product of p-nitroanilide (pNA) during 41 cycles (3 minute each) at 405 nm and 37°C. pNA is the end product stemming from PL cleaving the chromogenic substrate, pyro-Glu-Phe-Lys-p-nitroanilide hydroxychloride (Aniara, Biophen CS - 41(03), USA) (2.5 mg/mL). The substrate (40 µL of) was mixed with 150 µL of serum for the reaction to happen. As reviewed by Bastian and Brown (1996), the use of chromogenic substrate is sensitive and requires little sample preparation. Urokinase (4.5 µL of freeze dried urokinase from human kidney; 49.5 plough units) was added to the milk serum and substrate mixture to measure the total proteolytic activity of PL and PG. Urokinase is a PG activator that converts PG into PL (Walstra et al., 2005). The level of PG derived activity is then calculated

by the difference between total activity and PL activity as described by Abeni et al. (2008). The PL and total activities were measured in duplicates and 200 μL of plasmin buffer was pipetted into each of three wells serving as blank at each analysis. The PL activity and total activities were measured as change in absorbance during a specific time period using the linear part of the absorbance curves against time. Both PL and PG derived activities were measured in the same unit, i.e. the amount of PL or urokinase activated PG that causes a 0.001 change of absorbance at 405 nm during 1 minute at pH 7.4 and 37 °C under the experimental conditions.

3.6 Statistical analysis

Statistical analyses were performed using Minitab® 17.3.1 (Minitab, Inc., USA) to identify the effect of breed, milking systems and production month on PL, PG, TP and SCC. Normality tests were performed for PL, PG, TP and SCC. Analysis of variance (ANOVA) was carried out with the same software. Significance levels were identified at 95% individual confidence interval for mean values based on pooled standard deviation. Graphical illustrations were made using Minitab and SigmaPlot (Systat Software, San Jose, CA).

4 Results

4.1 Effect of breed on PL and PG of bulk milk

The bulk milk PL activity was significantly ($P < 0.05$) affected by breed (Figure 2). The farms having mixture of breeds had higher ($P < 0.05$) PL activities as compared to farms having Holstein, Swedish red and both Holstein, Swedish red and their crosses (Figure 2). No significant difference ($P > 0.05$) was observed in bulk milk PG derived activity as affected by breed.

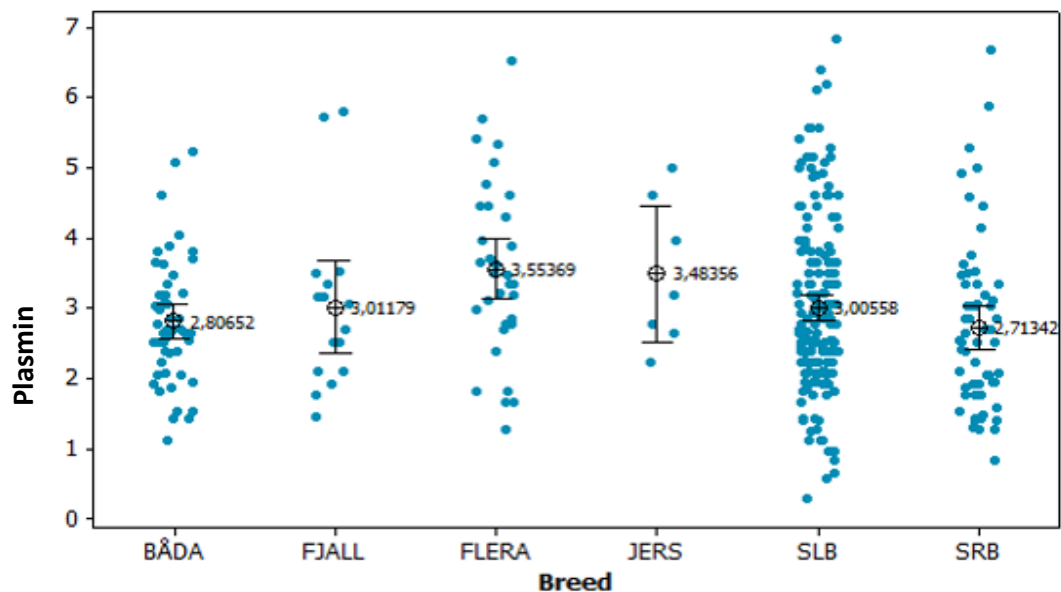


Figure 2. Scatter plot of variation of plasmin (PL) as affected by different breeds, each dot representing a PL value of one farm. Båda=Both Holstein, Swedish red and their crosses, Fjall=Mountain cows, Flera= Mixture of breeds, Jers=Jersey, SLB=Holstein, SRB=Swedish Red

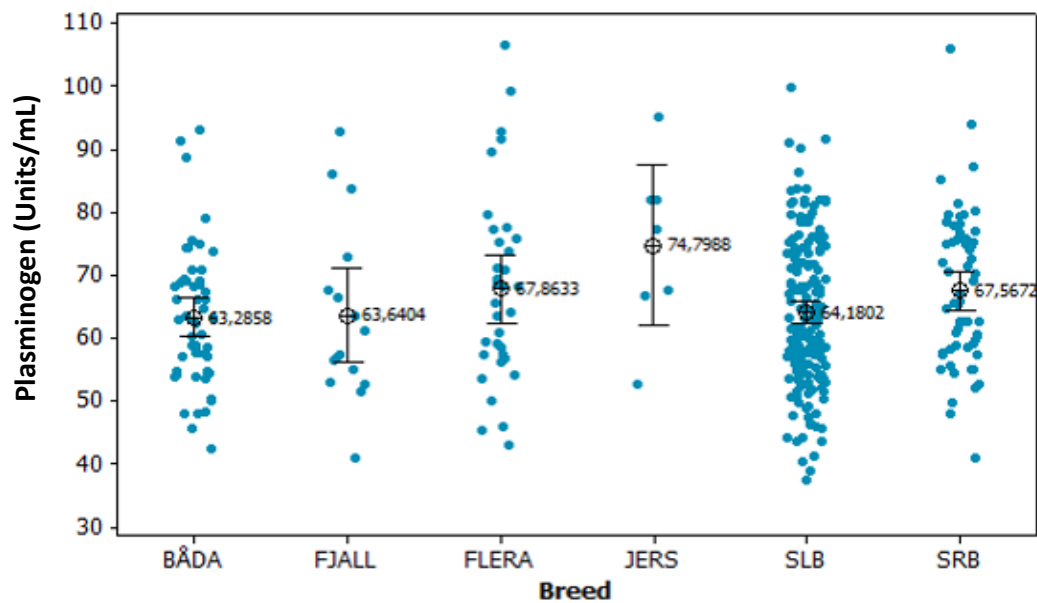


Figure 3. Scatter plot of variation of plasminogen (PG) as affected by different breeds, each dot representing a PG value of one farm. . Båda=Both Holstein, Swedish red and their crosses, Fjall=Mountain cows, Flera= Mixture of breeds, Jers=Jersey, SLB=Holstein, SRB=Swedish Red

4.2 Effect of breed on TP content of bulk milk

The mean TP content of different farms clustered according to the type of breed as shown in Figure 4. The mean TP content differed ($P < 0.05$) between the farms when they were clustered according to the type of breed. The highest ($P < 0.05$) TP value of 4.02 % was observed with farms having higher proportion of Jersey cows. The lowest TP value was observed for farms with Mountain breed (3.41%) and it differed ($P < 0.05$) from Holstein (3.49%), Swedish red (3.64%), Jersey (4.02%) and mixture of different breeds farms (3.64%).

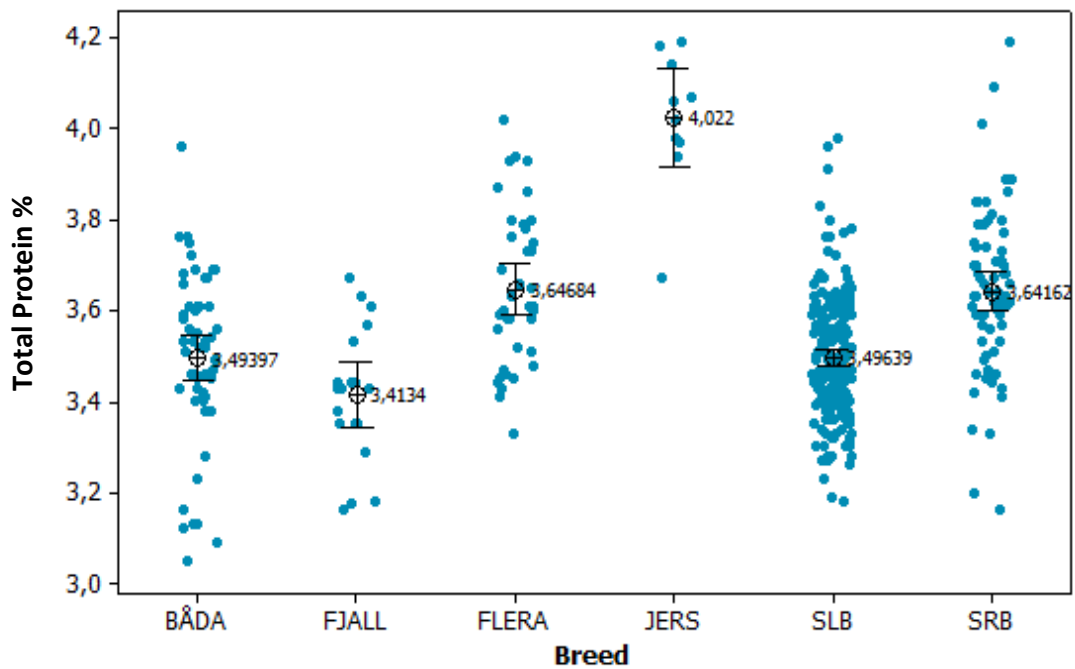


Figure 4. Scatter plot of variation of total protein percentage (TP %) as affected by different breeds, each dot represents a TP % of one farm. Båda=Both Holstein, Swedish red and their crosses, Fjall=Mountain cows, Flera= Mixture of breeds, Jers=Jersey, SLB=Holstein, SRB=Swedish Red

4.3 Effect of breed on SCC of bulk milk

The mean bulk milk SCC for each farm cluster is shown in Figure 5. The SCC of bulk milk varied ($P < 0.05$) among the farms clustered according to the predominant breed. Farms with majority of Swedish Red breed are reported to have lower ($P < 0.05$) SCC (121.67×10^3 cells / mL) than the other farms. The highest mean SCC of 223.53×10^3 cells /mL was reported for mountain cow farms and this value was higher ($P < 0.05$) than Swedish red farms (121.67×10^3 cells/ mL) and farms having both Swedish Red, Holstein and their crosses (172.67×10^3 cells / mL).

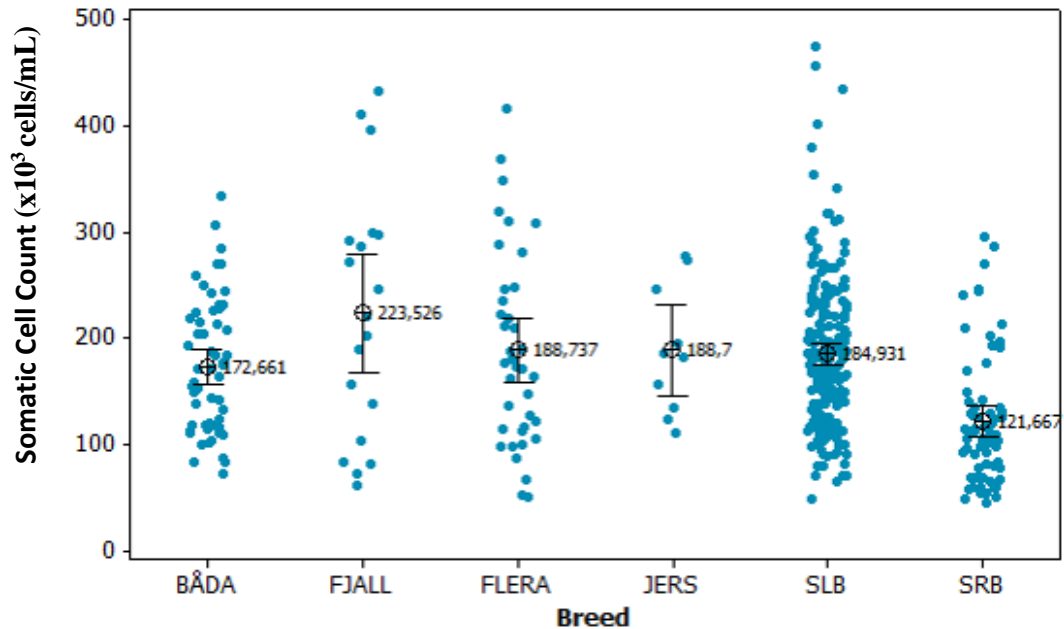


Figure 5. Scatter plot of variation of somatic cell count (SCC) as affected by different breeds, each dot represents a SCC value of one farm. Båda=Both Holstein, Swedish red and their crosses, Fjall=Mountain cows, Flera= Mixture of breeds, Jers=Jersey, SLB=Holstein, SRB=Swedish Red

4.4 Effect of milking system on PL and PG of bulk milk

The variations of bulk milk PL and PG derived activities according to the milking system are shown in Figure 6 and Figure 7, respectively. The milking system did not affect ($P > 0.05$) the PL and PG derived activities of the considered farms. However, there was a trend of having a higher mean PL activity (3.027 units/mL) in AMS compared to 2.959 units/mL of CMS. The mean PG derived activity showed to be higher in CMS (66.28 units/mL) compared to 63.69 units/mL in AMS.

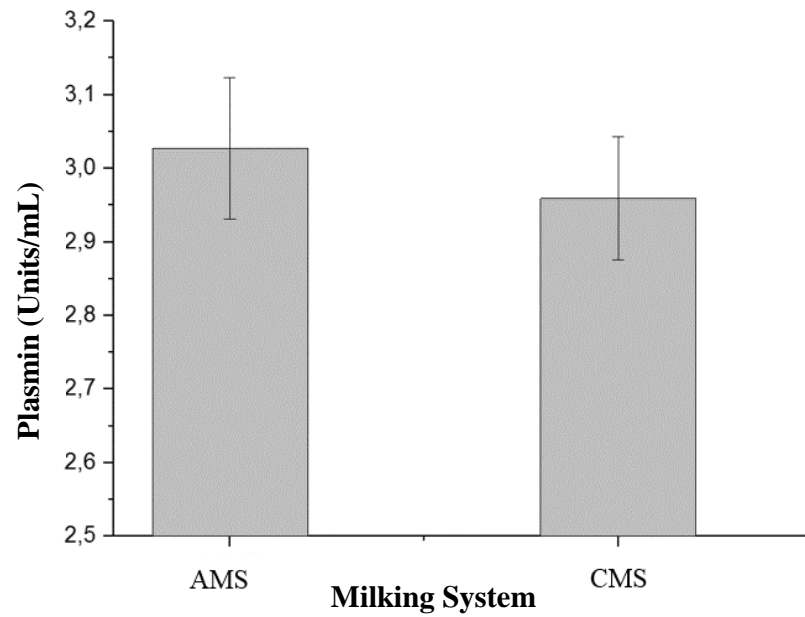


Figure 6. Variation of plasmin (PL) activity according to the milking system; AMS: Automatic milking system and CMS: Conventional milking system

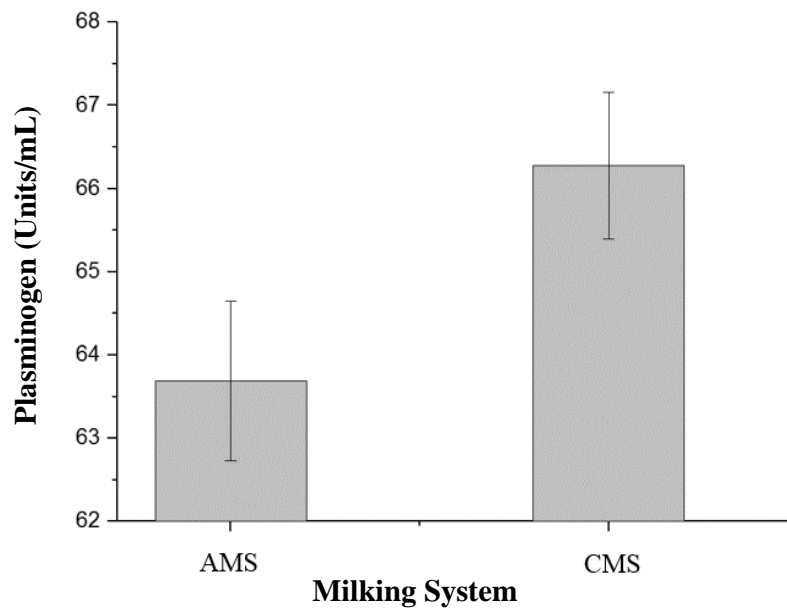


Figure 7. Variation of plasminogen (PG) derived activity according to the milking system; AMS: Automatic milking system and CMS: Conventional milking system

4.5 Effect of milking system on TP content of bulk milk

The TP content of bulk milk varied ($P < 0.05$) according to the milking system as shown in Figure 8. The bulk milk protein content of farms adapting CMS was higher ($P < 0.05$) (3.60%) than that of AMS farms (3.47%).

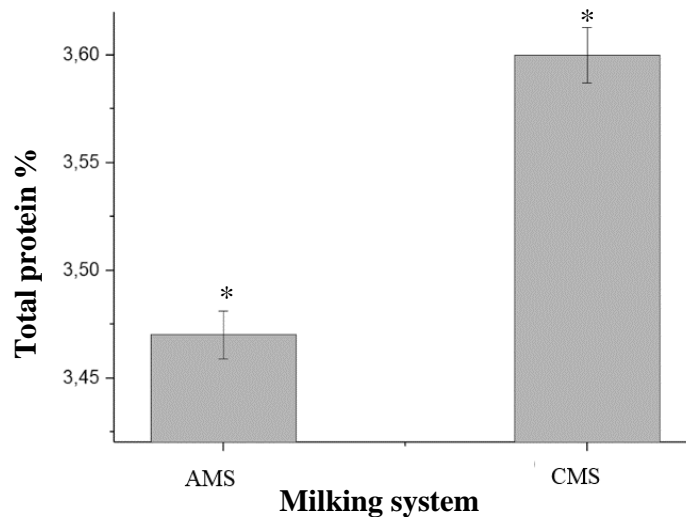


Figure 8. Variation of bulk milk total protein percentage according to the milking system; AMS: Automatic milking system and CMS: Conventional milking system, * Indicates the significance ($P < 0.05$)

4.6 Effect of milking system on bulk milk SCC

The average SCC in milk from farms with different milking systems is shown in Figure 9. The SCC of AMS farms was higher ($P < 0.05$) (193.54×10^3 cells / mL) than SCC of bulk milk from CMS farms (160.51×10^3 cells /mL).

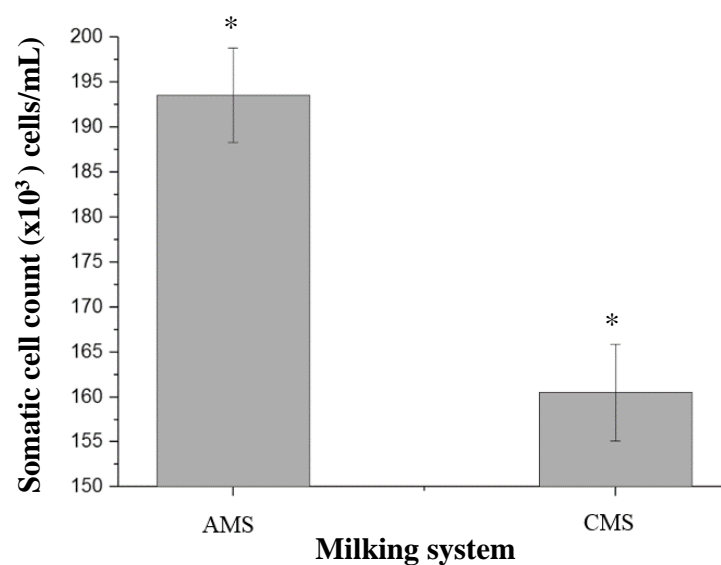


Figure 9. Variation of SCC according to the milking system AMS: Automatic milking system and CMS: Conventional milking system, * Indicates the significance (P < 0.05)

4.7 Variation of PL and PG with the production month

The variation in average (for all farms) monthly PL and PG derived activities of bulk milk between different production months is shown in Figure 10. The PL and PG derived activities of bulk milk varied over the year for the considered farms. The production month had no influence (P > 0.05) on the average PL and PG derived activities. The lowest (2.14377 units/mL) PL activity was observed in July and the highest (3.47367 units/mL) PL activity was observed in October. PG derived activity varied slightly over the year and the highest was observed in October (69.0032 units/mL) and November (77.0519 units/mL). Lower PG derived activity was observed in the summer months (June, July, August) and there was a trend of increasing PG derived activity until November, before it decrease towards February.

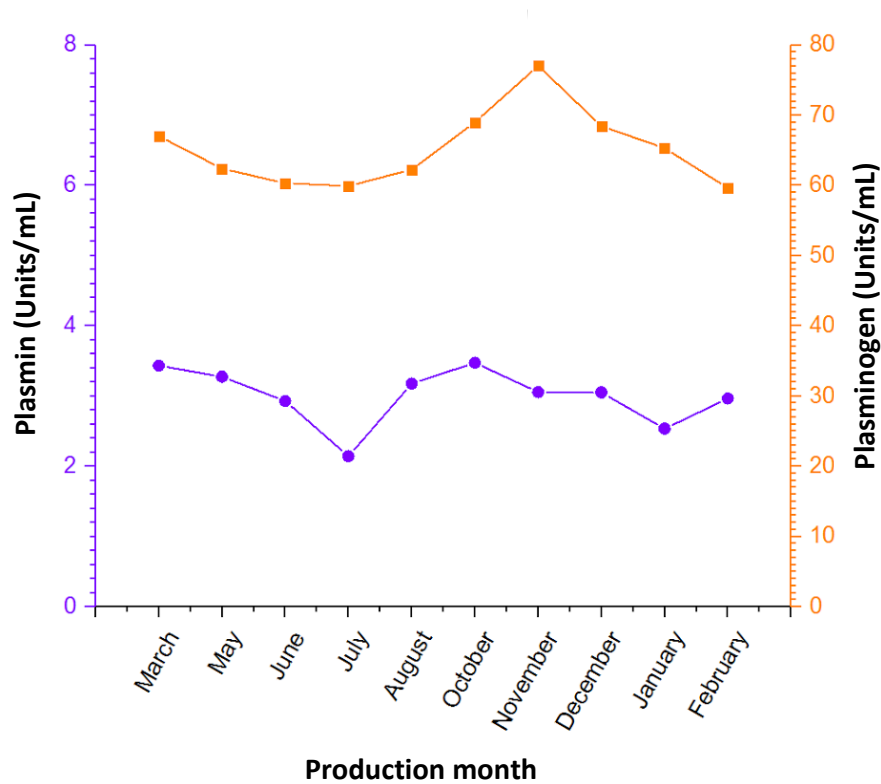


Figure 10. Variation of Plasmin (PL) and Plasminogen (PG) derived activities in bulk milk from March, 2016 to February, 2017

4.8 Variation of bulk milk SCC with the production month

The variation of bulk milk SCC according to the production month is shown in Figure 11. There was a slight variation of mean SCC of all farms according to the production month, but no clear trend over the months was observed. There was a trend of higher SCC in August (198.8×10^3 cells/mL) and the lowest in

March (152.3×10^3 cells/mL)

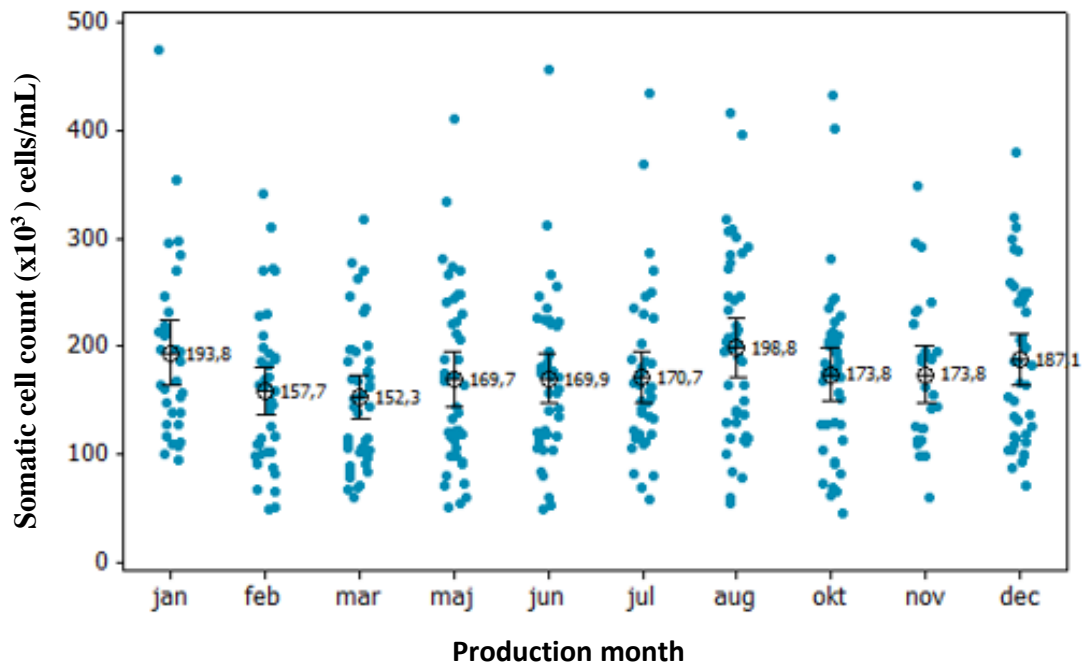


Figure 11. Variation of bulk milk Somatic Cell Count (SCC) from March, 2016 to February, 2017

4.9 Variation of bulk milk TP content with the production month

The variation of bulk milk TP content according to the production month is shown in Figure 12. The mean TP content varied ($P < 0.05$) with the production month. The mean TP content in October, November and December were higher ($P < 0.05$) than mean TP content in June, July and August.

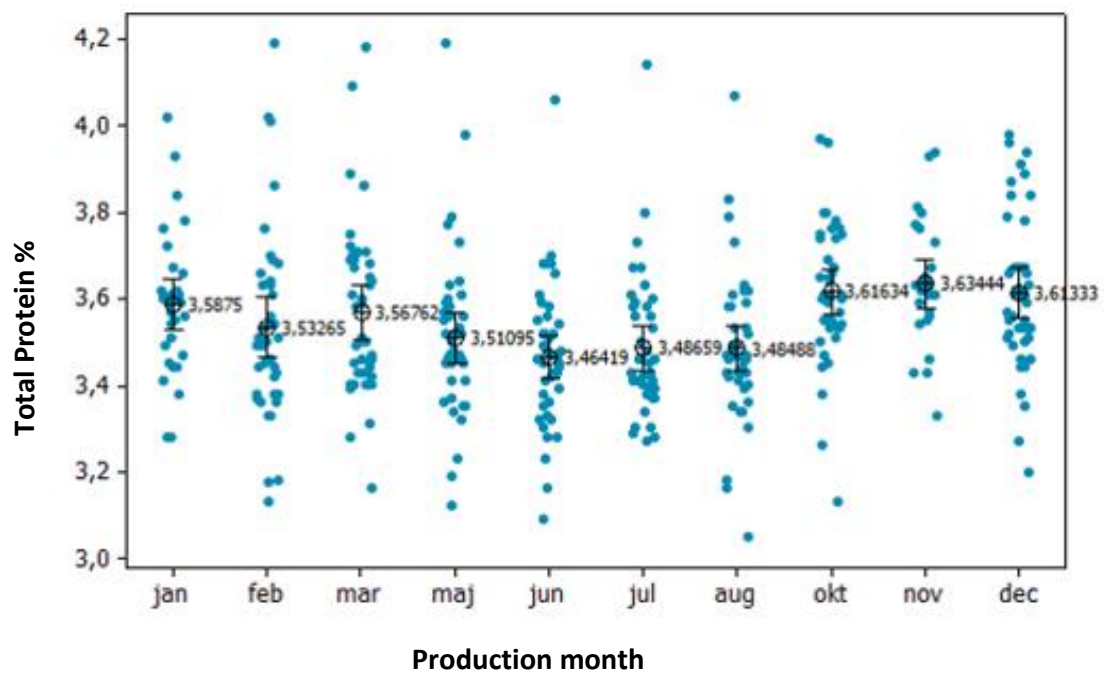


Figure 12. Variation of total protein percentage (TP %) in bulk milk from March 2016 to February, 2017

4.10 Correlations between PL/PG and TP content

There was no clear trend or correlation between neither bulk milk TP content, PL nor or PG derived activities respectively (Figure 13 and 14).

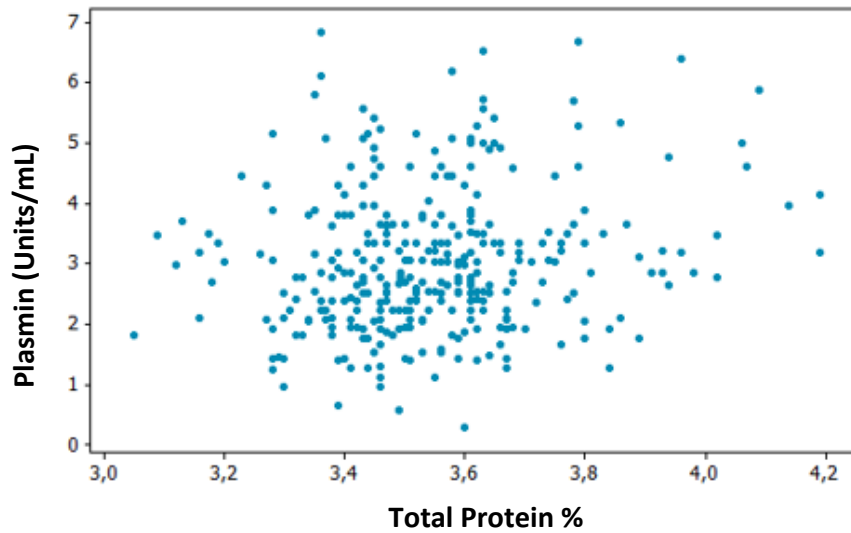


Figure 13. Correlation between plasmin (PL) and total protein percentage (TP %) of bulk milk

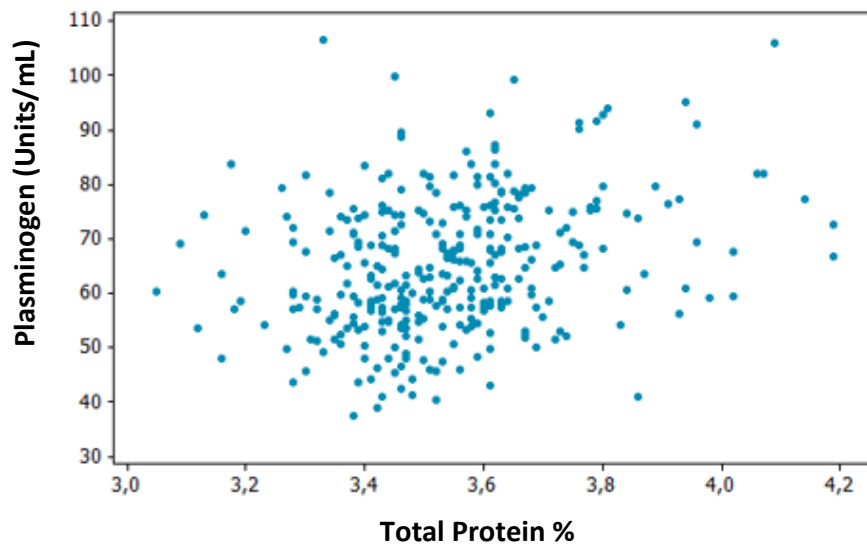


Figure 14. Correlation between plasminogen (PG) and total protein percentage (TP %) of bulk milk

4.11 Correlations between PL/PG and bulk milk SCC

There was no clear trend or correlation between neither bulk milk SCC, PL nor PG derived activities (Figure 15 and 16).

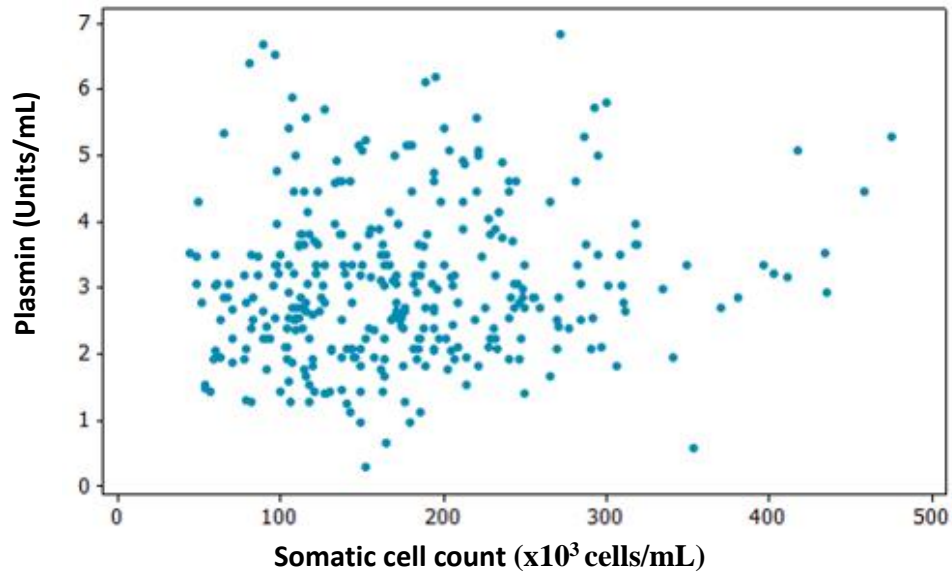


Figure 15. Correlation between Plasmin (PL) and Somatic Cell Count (SCC) of bulk milk

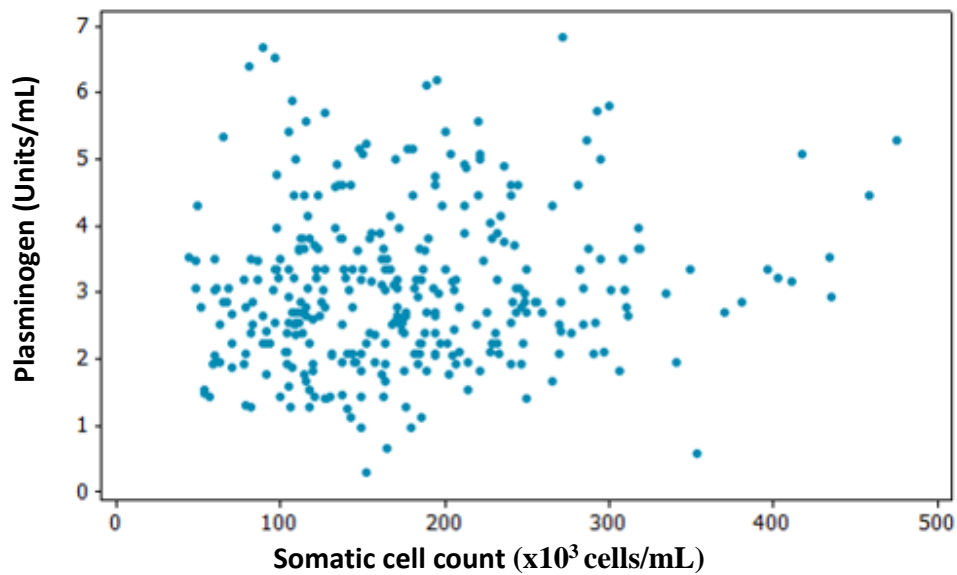


Figure 16. Correlation between plasminogen (PG) and somatic cell counts (SCC) of bulk milk

5 Discussion

5.1 Effect of breed on PL and PG of bulk milk

As shown in Figure 2 there was a significant breed effect on bulk milk PL activity. However breed effect was not significant for bulk milk PG derived activity (Figure 3). The PG result of the current study is in agreement with Fantuz et al. (2001) while the PL result is in contrast, Fantuz et al. reporting no breed effect of Holstein and Jersey cows on either PL or PG derived activities. In contrast, Richardson (1983) reported that PL and PG derived activities of milk varied according to the breed, where the Holstein Friesian breed in early and late lactation (0.27-0.53 mg/l) had a higher PL activity than that of Jersey cows (0.15-0.37 mg/l). Schaar, (1985) also reported that the PL activity varied according to the breed. However, the authors further reported that breed effect no longer existed when the adjustments were made for casein content. In the present study, it was not possible to observe the effect of breed on PG possibly due to the other variables (i.e. stage of lactation, parity, age), which are not included and considered in this particular study design. The present study was performed as a part of a bigger project, which is currently being implemented in close collaboration with the Åse Lundh group at SLU.

5.2 Effect of breed on TP content of bulk milk

The milk protein content of lowland cow breeds is around 3.5 % (w/w) and typically in the range of 2.3-4.4% (w/w) (Walstra et al., 1999). In agreement, the mean TP values of the current study ranged from 3.41% to 4.02%. According to this study, the breed had a significant influence on the bulk milk protein content. In agreement to with the current study, different protein percentages for different cow breeds were have been reported in several studies. Gustavsson et al. (2014)

compared the protein contents for three different breeds; (i.e. Swedish Red, Danish Holstein and Danish Jersey) and reported that the average protein content for the Swedish Red breed was 3.7 (g/100g). From the current study it was found that the bulk milk protein content of the Swedish Red breed was similar (3.64%) to the reported value. In the current study, the Jersey bulk milk contained significantly higher amount of proteins (4.02%) than the other breeds. This is in agreement to Linn, (1988) who reported that Jersey and Guernsey cattle generally have the highest percentages of TP compared to other breeds. Also Auld et al. (2004) reported higher protein composition in Jersey bulk milk compared to Holstein bulk milk.

5.3 Effect of breed on SCC of bulk milk

In the current study, the SCC of bulk milk significantly differed between farms based on the predominant breed. In agreement with our results, Gustavsson et al. (2014) reported an effect of breed on milk SCC. Further, the authors found that the Swedish Red breed had the lowest SCC with a mean of 54.1 ($\times 10^3$ cells /mL) compared to 110.3 and 113.1 ($\times 10^3$ cells /mL) for Danish Holstein and Danish Jersey, respectively (Gustavsson et al., 2014). This is in agreement with the current study, in which we observed a significantly lower SCC for the Swedish Red farms. However, the mean SCC of the Swedish Red farms in the current study was higher (121.67 $\times 10^3$ cells /mL) than the values reported by Gustavsson et al. (2014). Furthermore, the current study demonstrated that the milk from farms that are predominant with Swedish red cows are having better udder health and high milk quality in terms of SCC compared to the other farms.

5.4 Effect of milking system on PL and PG derived activities of bulk milk

The current study reported no significant differences in PL and PG derived activities as affected by the milking system. The results are in contrast to the findings of Abeni et al. (2008) and Johansson et al., (2017), who reported differences in PL and total activity of PL and PG with regard to the milking system. The authors reported lower PL and total activity with regard to the AMS than CMS. However, this is in contrast to the findings from the current study, with a trend of having lower PL in CMS than AMS. However, the PG derived activity of CMS was higher than that of AMS which is in agreement with the above authors. The differences in proteolytic enzymes may be attributed to the difference in milking intervals between the two types of milking systems. Longer

milking intervals in CMS makes more time available for PG to convert into PL. A study done by Sorensen et al. (2001) concluded that thrice a day milking resulted in higher PG to PL ratio than twice a day milking. Further, the authors argued that thrice a day milking (increased milking frequencies) resists the leakage of proteolytic enzymes from serum to milk by helping to maintain the integrity of epithelial tight junctions. This might be the reason to report lower PG derived activity in AMS than CMS as observed in the current study.

5.5 Effect of milking system on TP content of bulk milk

The current study reported significantly higher TP content in bulk milk with CMS than AMS. In agreement with the current study, Johansson et al., (2017) reported that protein percentage was lower in AMS bulk milk than CMS. However, as reviewed by Linn, (1988) the effect of milking system and milking frequency on protein percentage is minor. Even though the CMS and AMS differ in terms of milking frequency, Rogers and Stewart (1982) reported that the protein content of milk is not affected by milking frequency unless the interval between successive milking exceeds 16 hours. Abeni et al. (2008) also reported no difference in protein content between two milking systems. O'Brien et al. (2002) reported a correlation between the milk protein content and milking frequency. According to the authors, once daily milking had higher protein content than twice daily milking. The finding suggests that increased milking intervals result in higher protein contents of milk. This might be the reason for containing higher amount of TP in CMS compared to AMS in the current study. Furthermore, the effect of milking system has a clear influence from breed, as the majority of the representative breed type differs between the types of milking system. Hence, it is not possible to draw a clear connection to the individual effect of milking system or breed effect on protein content rather than holistic effect of both main parameters.

5.6 Effect of milking system on SCC of bulk milk

The current study reports significantly high SCC in bulk milk from farms with AMS. This is in agreement with Rasmussen et al. (2002) and Johansson et al., (2017). According to Rasmussen et al. (2002) shifting from CMS to AMS lead to an increase of the SCC of bulk milk. However, the authors concluded that high SCC in AMS can be overcome by introducing self-monitoring system and paying attention for cows with clinical mastitis. As reported by Johansson et al. (2017) the SCC of milk from AMS was 26% higher than SCC of milk from CMS. In contrast

to the current study, Abeni et al. (2008) reported no difference in SCC between two milking systems. The possible reasons for observing the differences in SCC in terms of milking systems in the current study may be attributed to other factors (i.e. breeds, lactation, management practices, cow health and welfare status), which were not included in analysing the effect of milking system on SCC. In the present study factors are often of a multidimensional nature and factors that have not been included, yet being related to the SCC, could be the reason for the higher SCC in AMS than CMS.

5.7 Variation of PL and PG derived activities with the production month

The differences in PL and PG derived activities between production months were not significant, yet, activities showed slight trends with respect to their variation. A study by Karlsson et al. (2017) reported that the PL activity of Swedish raw milk ranged from (minimum) 2.22 Units/mL to (maximum) 4.22 Units/mL, and according to the current study, the minimum PL activity of bulk milk was 2.14 Units/mL and maximum was 3.47 Units/mL. Similar to the findings from the current study, Karlsson et al. (2017) concluded that the effect of season on raw milk proteolytic activity was not significant. Benslimane et al. (1990) reported variations of PL and PG derived activities according to the month of the year. These authors reported the highest PL activity in June and the lowest in September with regard to the bulk milk. The highest PL value in the current research was for October while the lowest was for July. Benslimane et al. (1990) reported the highest PG derived activity in October and the lowest in September for bulk milk. In agreement, in the current research maximum PG derived activity was reported in October and November. According to Nicholas et al. (2002), the PL and PG derived activities are subject to change according to the quality and quantity of the feed as affected by the seasons. Hence, the changes of PL and PG derived activities according to the production month might be attributed to the changes in temperatures as well as changes in feeding according to the season. However, the reason for not observing significant differences may be because the differences between farms in terms of the production month that they switch from winter feeding to summer feeding and vice versa. Further, Karlsson et al. (2017) discussed that the probable reasons for not observing a clear effect of season on PL and PG derived activities was due to non-seasonal calving patterns and low number of psychrotropic bacterial content in milk.

5.8 Variation of SCC of bulk milk with the production month

There was no clear trend or significant variation in SCC according to the month. The result is in agreement with a study done with Holstein dairy cows (Bernabucci et al., 2015). The authors concluded that the effect of season on SCC was mild. However, the authors observed that SCC was higher in summer milk than winter and spring milk (Bernabucci et al., 2015). In contrast, Erdem et al. (2007) reported significantly higher SCC (log SCC) in summer milk from Holstein cows than that of other seasons. The authors concluded that higher SCC in summer may be attributed with the thermal stress and the higher number of pathogenic organisms during summer season. This might be the reason for reporting highest SCC in August in the current study. However, no clear pattern of variation was observed and this may be due to the effect of other factors that affect SCC other than the environmental temperature and seasonal variation associated factors.

5.9 Variation of TP content of bulk milk with the production month

The TP content of bulk milk significantly varied according to the production month. The mean TP content was significantly higher in October, November and December than in June, July and August. This is in agreement with Ng-Kwai-Hang et al. (1982) and they concluded that TP, casein and serum proteins had an increasing trend towards December from July. Another study conducted by Bernabucci et al. (2015) determined the effect of summer season on milk protein of Holstein dairy cattle and they concluded that the milk protein fractions were lower in summer milk than in winter milk. According to Walstra et al (1999) a low protein diet can affect the milk to have lower protein content. The changes in TP content in the current study may be attributed with the changes in diet. Also the low TP content of summer milk may be attributed with high temperatures during summer. According to Bernabucci et al. (2015), the hot weather in summer cause to reduce the milk casein concentration that may contribute to reduce the total milk protein content.

5.10 Correlations between PL/PG and TP content

In contrast to several other studies, no correlations between PL activity, PG derived activity and TP content was observed in the current study. As reviewed by Ismail and Nielsen (2010), the components of PL system interact together with other milk components such as caseins and whey proteins. According to Walstra et

al. (1999) considerable portion of PL is associated with the casein micelles. A relationship between α s-1 and β caseins fractions with PL activity was reported by de Vries et al. (2016). The authors reported decrease of α s-1 and β -casein fractions when PL activity was increase. Johansson et al., (2017) also reported a negative correlation between total proteolysis and β -casein fraction ($P < 0.05$). A link between PG to γ -caseins was reported by Benslimane et al. (1990) in the study where the variations of PL and PG with seasons and lactation in Montbeliard cows' milk were investigated. According to the authors, PG derived activity of milk was positively correlated with the γ -caseins level of milk. But milk with high proportion of γ -caseins did not exhibit higher PL activity. However, Bastian et al. (1991) observed that the PL activity was not influenced by the milk protein content. It is in agreement with the current study which reports no correlation between bulk milk PL activity, PG derived activity and TP content. Furthermore, this may be due to many inhomogeneous and unknown variabilities among the farms that may affect the TP content and PL and PG derived activities.

5.11 Correlations between PL/PG and bulk milk SCC

Correlations between bulk milk SCC and PL/PG were not observed in the current study and it is in contrast to several other studies. It is known that milk with a high SCC often shows a higher PL activity. This is due to presence of a promoter in leucocytes that catalyses the conversion of PG to PL (Walstra et al., 1999). A study done by Polidori et al. (1999) using Holstein and Jersey cows to investigate the PG activation system and its relationship to milk production and composition concluded that PG activation system had a positive correlation with the SCC. Moreover, Chavan et al. (2011) revealed that increased levels of PAs cause the mastitis milk to exhibit a higher PL activity. It would be the similar reason for the increased PL activity of milk having higher SCC. A clear increment of PL and PG with increasing SCC was reported by Politis et al. (1989a). According to the authors, increasing SCC from below 250, 000/mL to more than 1,000,000/mL corresponded to an increase in PL activity by 105% and PG by 74%. Johansson et al., (2017) also reported a positive correlation between SCC and total proteolysis ($P < 0.01$). However, the reason for not observing a clear correlation between SCC and PL/PG in the current result may be due to other inhomogeneous and unknown variables between farms that affect the SCC and PL, PG of bulk milk.

6 Conclusions

The bulk milk PL activity was significantly affected by the breed. Milk from farms having mixtures of breeds had significantly higher PL activities as compared to farms having Holstein, Swedish Red and both Holstein, Swedish Red and their crosses. However, bulk milk PG derived activity had very little influence from breed. Milk from farms having Mountain breeds had significantly lower protein content than Holstein, Swedish red, Jersey farms and farms having mixture of breeds. Jersey breed resulted in higher milk protein content than other breeds. Thus, protein content of bulk milk was influenced by the predominant breed in the farms. Milk from farms with a majority of Swedish Red breed had significantly lower SCC than milk from other farms, while Mountain breeds had significantly higher SCC compared to Swedish Red and farms having both Swedish Red, Holstein and their crosses. The milking system did not significantly affect the PL and PG derived activities of the considered farms. CMS farms had significantly higher bulk milk protein content than AMS farms. AMS farms had significantly higher SCC than CMS farms. The production month showed no significant influence on the average PL and PG derived activities or SCC. The mean TP content significantly varied with the production months, with significantly higher TP content in October, November and December than June, July and August. No clear correlations were found between TP, SCC and PL, PG. The variabilities between farms which were not included here may be the reason for not showing the correlations. The effects of PL and PG as a function of breed, milking system and production month may be not optimally visible due to the interrelated and heterogeneous sampling techniques adopted for this specific individual study from the larger project. Thus, it is recommended to perform future studies by isolating the factors in consideration of current study and evaluate those independently by eliminating the holistic approach.

7 Acknowledgements

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Appendix 1

Can the activity of plasmin system controlled at your farm?

Dairy proteins are important for processing and determining the final product characteristics. Approximately 80% of dairy protein is casein. Thus any potential influence on caseins, may impart the processability of raw milk in to the final product and thereby detrimentally affect the final product quality. It is highly important to maintain the appropriate balance between casein and casein degradation enzymes in milk. Plasmin system is one of the most important, indigenous proteolytic enzyme system found in bovine milk, which is of interest for many dairy producers and processors. Plasmin is the major component of plasmin system and plasminogen is its zymogen form. Appropriate balance of plasmin in bulk milk is known to be beneficial in producing long ripened cheeses and therefore, this study aimed to evaluate the variation of plasmin, plasminogen, total protein content and somatic cell count in different farms that deliver milk to dairies for cheese production in northern Sweden. Mainly three variables were considered for the study to look how those can affect the aforementioned bulk milk parameters. Opted variables were cow breed, milking system and production months. At the end of the current study, it was expected to develop the fundamental understanding of how the plasmin activity, plasminogen derived activity, total protein and somatic cell count in bulk milk is influenced by the considered variables. Further, it was speculated to develop the linkage between plasmin activity, plasminogen derived activity, total protein content and somatic cell count to develop basic knowledge for optimizing the production of raw milk that is indented for long ripened cheese production in northern Sweden. Bulk milk samples were collected from those selected farms for a period of one year and milk analysis were performed at SLU, Uppsala and Eurofins Laboratory.

There is established knowledge that plasmin activity and plasminogen derived activity are influenced by the on farm factors such as lactation number, breed, stage of lactation, milking frequency, milking system, stocking density of cows and diet. However, the specific knowledge on how the combination of milking system, breed and production months influence the plasmin activity, plasminogen derived activity, total protein content and somatic cell count in Swedish bulk milk is unknown. Plasmin system can be altered by many other factors such as, lactation number, stage of lactation, diet, stocking density of cows, processing of milk, storage of milk and mastitis conditions of the cows. Therefore, plasmin system is under the influence of multifactors, which make it difficult to study. However, it is

crucial to understand the effects of all involved factors in scientific perspectives for better controllability of the final product quality as well as storability of the bulk milk.

The results from current study demonstrated that plasmin activity is affected by the breed. However, the plasminogen derived activity was not influenced by the breed. The breed had an impact on total protein content of the bulk milk, where farms having majority of Jersey breed was reported to contain higher amount of total protein content compared to considered other farms (farms having majority of Holstein, Swedish Red, mountain breeds, both Holstein, Swedish Red and their crosses and mixture of breeds). Further, somatic cell count was lowest in farms having majority of Swedish Red cows compared to the other farms. There was no significant effect of milking system on plasmin activity and plasminogen derived activities. The study concluded that the milk from farms adapting conventional milking system had higher total protein content and lower somatic cell count than milk from farms adapting automatic milking system farms. Therefore, this study suggested that there are visible clues concerning the impact of milking system on protein content and somatic cell content. The current study did not found any significant effect from production months on plasmin activity, plasminogen derived activity and somatic cell count. However significantly higher protein content in bulk milk was reported from winter months (October, November and December) compared to summer months (June, July and August). Further, present study reported that there was no clear linkage between total protein and somatic cell count to plasmin activity and plasminogen-derived activity. It is speculated that the project generated updated knowledge regarding the influence to the plasmin activity, plasminogen derived activity, total protein content and somatic cell count by the aforementioned factors under the light of other unconsidered farm variables. Thus, the current study suggested to perform future studies by isolating the factors in consideration in the present study and evaluate those independently by eliminating the holistic approach to draw clear connections and links between the studied parameters. Moreover, it is suggested to analyse the total proteolytic activity and the detailed casein profile of bulk milk to investigate the effects of studied variables and their correlations.